(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
6 November 2003 (06.11.2003)

PCT

(10) International Publication Number
WO 03/091452 A1

(51) International Patent Classification[7]: C12Q 1/68, C12N 15/10

(21) International Application Number: PCT/IB03/01553

(22) International Filing Date: 22 April 2003 (22.04.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0209382.1 24 April 2002 (24.04.2002) GB

(71) Applicant *(for all designated States except US)*: QIAGEN AS [NO/NO]; Frysjaveien 40, N-0884 Oslo (NO).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: DEGGERDAL, Arne [NO/NO]; Engelsrudlia 59, N-1385 Asker (NO). SKAGESTAD, Vidar [NO/NO]; Kitty Kiellandsvei 15G, N-1344 Haslum (NO).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/091452 A1

(54) Title: ISOLATING NUCLEIC ACID

(57) Abstract: A process for isolating nucleic acid from a nucleic acid- containing biological sample, which comprises: (a) providing magnetic particles capable of binding nucleic acid; (b) contacting the sample with the magnetic particles so as to bind the nucleic acid thereto; (c) forming an aggregate of the magnetic particles by the application of a magnetic field, which aggregate comprises magnetic particles with the nucleic acid bound thereto and contaminants from the biological sample; (d) contacting the aggregate with a washing solution to disperse the aggregate and release the contaminants therefrom; and (e) separating therefrom the magnetic particles with the nucleic acid bound thereto; wherein the aggregate is formed in the presence of an amount of a water-miscible lower alcohol sufficient to facilitate dispersion of the aggregate and release of the contaminants therefrom when washed in step (d), with the proviso that, when the magnetic particles are silica-based magnetic glass particles provided with the water-miscible lower alcohol, the water-miscible lower alcohol is part of an aqueous mixture which further comprises a salt.

ISOLATING NUCLEIC ACID

Field of the Invention

The present invention relates to a process for isolating nucleic acid from a nucleic acid-containing sample, and to a kit therefor.

Background to the Invention

Procedures involving nucleic acids such as DNA and RNA continue to play a crucial role in biotechnology. Nucleic acid detection and manipulation including hybridisation, amplification, sequencing and other processes generally require nucleic acid to have been isolated from contaminating material. Where a nucleic acid-containing sample is a biological sample, contaminating material may include proteins, carbohydrates, lipids and polyphenols. Accordingly, a variety of approaches have hitherto been used in the isolation of DNA or RNA.

Early methods of isolating nucleic acid involved a series of extractions with organic solvents, involving ethanol precipitation and dialysis of the nucleic acids. These early methods are relatively laborious and time-consuming and may result in low yield. Isopropanol may also be used in the precipitation of the nucleic acid. Precipitation of the nucleic acids generally requires alcohol at a very high concentration, preferably 50% v/v or above.

An alcohol precipitation method is described in US5523231. Nucleic acid is precipitated by highly concentrated alcohol in the presence of magnetic beads. According to this

disclosure, use of an alcohol such as ethanol or isopropanol at around 70% (v/v) causes nucleic acid to precipitate around the magnetic beads but not to bind to the bead. The precipitate can be separated from supernatant by the application of a magnetic field.

US5234809 describes a procedure to isolate DNA from biological samples which uses a chaotropic agent together with a silica based nucleic acid binding solid phase. Guanidine hydrochloride at pH 3 to 5 or guanidine thiocyanate at higher pH, combined with other salts, is used as the chaotropic agent. After binding of the DNA to the solid surface, the solid phase may be washed with the chaotropic agent to remove any biological contamination followed by treatment with 70% ethanol to remove the chaotrope. The DNA is eluted using water.

A variant on this methodology is described in US6027945. Here, a method is described which also uses a silica-based nucleic acid binding solid phase in the presence of a chaotrope to isolate nucleic acid. According to this method, the silica-based solid phase is magnetic, thereby facilitating separation of the solid phase containing the target nucleic acid from the liquid phase containing contaminants upon application of a magnetic field.

In one commercially-available product from Roche, a "MagNa Pure LC Total Nucleic Acid Isolation Kit" is provided to isolate DNA or RNA using magnetic glass particles in the presence of a chaotropic salt and Proteinase K. The magnetic glass particles are provided as a suspension in

isopropanol. There is a need to use several washing steps and the Proteinase K to purify the nucleic acid.

WO96/18731 also uses magnetic particles to bind nucleic acid. In this disclosure the magnetic particles are polystyrene-based and polyurethane-coated and a detergent is used instead of a chaotrope.

Liquid handling instruments, which may be operated automatically, are commonly used to isolate nucleic acid. Such instruments frequently use magnetic beads for isolating the nucleic acid and there exist a number of different ways in which the magnetic beads are separated from liquid phase during operation of the nucleic acid isolation process. Generally, the liquid handling instrument has a reaction zone which may comprise, for example, a tube, channel or well, which may or may not communicate with an outlet, usually by means of a narrower portion such as a tip. In one arrangement, a magnet is placed outside of the reaction zone so that magnetic particles in the reaction zone are collected in an aggregate inside the reaction zone. In a second arrangement, separation and collection of the aggregate takes place inside the tip by attaching a magnet outside of the tip. In a third arrangement, where the reaction zone is a tube or well, a plastics coated magnetic rod may be placed inside the tube or well in order to separate the magnetic particles from the supernatant.

Wherever magnetic particles are used to bind nucleic acid in any of the above isolation processes, especially those

in liquid handling instruments, application of the magnetic field gives rise to an aggregate which contains not only nucleic acid bound to the particles but also contaminating material. The constitution of this contaminating material will depend upon the biological source material from which the nucleic acid is being isolated. Protein is often a major contaminant. The magnetic particles and bound nucleic acid have to be separated from the contaminants by washing. However, here a problem arises. First of all, the aggregate is difficult to disperse because the constituents of the aggregate tend to clump together, often presenting a mass of tightly adhered material. Secondly, if the contaminants are not properly removed, the final isolated yield of nucleic acid is decreased. These problems arise in both manual and automated arrangements. Attempts to disperse the aggregate have met with limited success. Physical agitation of the aggregate, for example by flowing washing solution at raised pressure across the aggregate, can be used to disrupt the aggregate to some extent. Such a step is not particularly efficient, being time-consuming and not always capable of completely dispersing the aggregate. Many protocols require the additional step of adding Proteinase K in order to reduce the viscosity of the aggregate by hydrolysing the protein portion of the contaminants. This has the disadvantage of being an extra step which increases the time required for separation and also increases the cost. It is also often the case that an additional biological contaminant, Proteinase K in this case, is undesirable where high purity nucleic acid samples are required.

Summary of the Invention

In accordance with the present invention there is provided a process for isolating nucleic acid from a nucleic acid-containing biological sample, which comprises:

(a) providing magnetic particles capable of binding nucleic acid;

(b) contacting the sample with the magnetic particles so as to bind the nucleic acid thereto;

(c) forming an aggregate of the magnetic particles by the application of a magnetic field, which aggregate comprises magnetic particles with the nucleic acid bound thereto and contaminants from the biological sample;

(d) contacting the aggregate with a washing solution to disperse the aggregate and release the contaminants therefrom; and

(e) separating therefrom the magnetic particles with the nucleic acid bound thereto; wherein the aggregate is formed in the presence of an amount of a water-miscible lower alcohol sufficient to facilitate dispersion of the aggregate and release of the contaminants therefrom when washed in step (d).

It is preferred that, when the magnetic particles are silica-based magnetic glass particles provided with the water-miscible lower alcohol, the water-miscible lower alcohol is part of an aqueous mixture which further comprises a salt.

Hitherto it has not been realised that forming the aggregate in the presence of a water-miscible lower alcohol facilitates dispersion of the aggregate and release of the

contaminants therefrom. This has the advantage that the need to use Proteinase K to digest the sample before the washing step (d) is reduced or eliminated altogether. There is a further advantage that the washing step may be performed more efficiently. In addition, it is found that the total isolated yield may be increased thereby allowing a greater initial load of sample to be used for the same amount of magnetic particles.

In a second aspect, the present invention provides a kit for isolating nucleic acid from a nucleic acid-containing biological sample, which kit comprises:

(a) magnetic particles capable of binding nucleic acid;

(b) a source of a water-miscible lower alcohol; and

(c) a washing solution for use in the presence of the water-miscible lower alcohol for dispersing an aggregate comprising the magnetic particles with nucleic acid bound thereto and contaminants from the biological sample.

Preferably the magnetic particles are not the source of the water-miscible lower alcohol when the magnetic particles are silica-based magnetic glass particles unless (i) no Proteinase K is present in the kit, or (ii) the water-miscible lower alcohol is part of an aqueous mixture which further comprises a salt.

It is preferred that substantially no Proteinase K is present to digest protein in the process so as to avoid the disadvantages of the use of this biological additive.

The water-miscible lower alcohol may be provided at any one or more points in the process provided that there is a sufficient amount in the step of forming the aggregate to be effective when the aggregate comes to be washed. In one arrangement, it is convenient to provide the water-miscible lower alcohol with the magnetic particles, for example as a suspension in the alcohol, preferably as an aqueous mixture thereof, which aqueous mixture may further comprise a salt. The salt may be organic or inorganic and inorganic salts such as alkali metal chlorides, for example sodium or lithium chloride, are preferred. The aqueous phase may include a salt in the range 1mM to 10M, preferably in the range 0.1M to 10M, more preferably in the range 0.4M to 8M. The ratio of alcohol:water may be approximately 2:1 mixture. Where the sole source of water-miscible lower alcohol is the liquid phase suspending the magnetic particles, it is thought that some of the alcohol remains associated with the magnetic particles in the void volume of the aggregate which is formed in the process.

Additionally, or alternatively, at least a part of the water-miscible lower alcohol may be provided in a lysis step used to treat the biological sample, especially where the biological sample comprises a cellular sample. Typically, the lysis step comprises subjecting the biological sample to conditions to lyse the sample, which conditions may include conventional use of a detergent and/or chaotrope. The water-miscible lower alcohol may be present in the lysis step in an amount in the range 1 to 40%, preferably 3 to 40%, most preferably 5 to 30%. The water-miscible lower alcohol may be provided as part of a

pre-formulated lysis solution or may be added during the lysis step.

Additionally or alternatively, at least a part of the water-miscible lower alcohol may be provided when the sample is contacted with the magnetic particles. The alcohol may be added shortly before contact or at the point of contact or may be part of a binding solution or even part of a combined lysis and binding solution. The water-miscible lower alcohol may be present in an amount in the range from 1 to 40%, preferably 1 to 20%, more preferably 5 to 10% in this step.

Additionally or alternatively, the water-miscible lower alcohol may be provided at the time when the aggregate is formed or shortly before.

Among the water-miscible lower alcohols useable in the present invention are included linear or branched chain alcohols with up to 5 carbon atoms. Of these alcohols, methanol is not preferred because of its potentially deleterious affects on human health. Ethanol, isopropanol and n-propanol are particularly preferred as being readily available and effective in the invention.

In the step of contacting the aggregate with a washing solution, further water-miscible lower alcohol may be provided with the washing solution. This is found to be particularly effective in dispersing the aggregate provided that the aggregate has been formed earlier in the presence of the water-miscible lower alcohol as well. The further

water-miscible lower alcohol used in the washing step may be the same as or different from the water-miscible lower alchohol used earlier in the process. The alcohol may be present in an amount in the range from 1 to 90%, more preferably 5 to 70%, most preferably from 10 to 40% v/v with respect to the washing solution.

The washing step may be repeated by removing supernatant, adding fresh washing solution and separating the magnetic particles from the supernatant by application of a magnetic field for a second time.

The washing solution according to the invention may comprise a chaotrope to facilitate washing. This chaotrope may be the same as or different from the chaotrope used in any preceding lysis or binding step.

The chaotrope generally comprises a chaotropic ion provided at a concentration sufficiently high to cause the nucleic acid to lose its secondary structure and, in the case of double-stranded nucleic acids, to melt. Chaotropes are thought to disrupt hydrogen-bonding in water so as to make denatured nucleic acid more stable than its undenatured counterpart. The chaotrope typically comprises a guanidinium salt, urea, or an iodide, chlorate, perchlorate or (iso)thiocyanate. Preferred chaotropes include guanidinium thiocyanate, and guanidinium hydrochloride.

The concentration of chaotrope typically present when contacted with the sample is in the range 2M to 8M.

Where a chaotrope is present in the washing solution, the process may further comprise a washing step to remove that chaotrope. Typical washing steps include contacting the magnetic particles to which nucleic acid is bound with an alcohol-containing solution such as ethanol in an amount of at least 70% v/v.

The nucleic acid-containing sample typically comprises a biological sample such as a cellular sample. The biological sample may or may not need to be pretreated, depending on its structure. For example, in the case of plant or fungal cells or solid animal tissue, pretreatment would be required as is known in the art. Samples stored in the form of a solid phase such as a paraffin section may also need pretreatment. Samples may be from foodstuffs, environmental samples or clinical samples and may contain prokaryotic or eukaryotic cells or other moieties such as mycoplasmas, protoplasts or viruses. Blood products are an important area for nucleic acid isolation and the present invention is particularly applicable to whole blood and other blood products such as plasma, serum and buffycoat.

The nucleic acid to be isolated may be DNA, RNA or a modified form thereof. Where the nucleic acid is DNA, this may be ds or ss DNA. Where the nucleic acid is RNA, this may be rRNA, mRNA or total RNA.

The magnetic particles must be capable of binding nucleic acid but are not limited to any specific material. Various materials are now known and these include silica-based materials such as those described in US5234809, polymeric

materials including latex and polystyrene-based materials such as those described in WO96/18731 and other materials such as glasses.

Typical materials for use in magnetic particles include magnetic metal oxides especially the iron oxides. Useful magnetic oxides include iron oxides in which, optionally all or a part of the ferrous iron thereof is substituted with a divalent transition metal such as cadmium, chromium, cobalt, copper, magnesium, manganese, nickel, vanadium and/or zinc. Silica-based magnetic particles useful in the present invention include those described in US6027945 and US5945525. These silica-formed particles generally require the presence of a chaotrope to be effective in binding nucleic acid.

Particularly preferred magnetic particles comprise polymer magnetic particles in which the magnetic component thereof is substantially uniformly distributed. Such particles are described in UK patent application no. 0116359.1, filed on 4th July 2001. Such particles are also described in UK patent application no. 0116358.3, also filed on 4th July 2001. The contents of the applications are hereby incorporated by reference.

In accordance with GB0116359.1 a process is provided for the preparation of polymer magnetic particles, which comprises:

(a) providing a water phase containing magnetic components homogeneously dispersed therein;

(b) wherein the water phase is contacted with or further contains a polymerisable metal-containing or organic monomer which is soluble in the water phase; and

(c) polymerising the monomer in the presence of the magnetic components so as to form polymer magnetic particles in which the magnetic components are substantially uniformly distributed;

wherein at least a part of the polymerising step (c) is carried out in a water-in-oil emulsion in which the water phase containing the magnetic components homogeneously dispersed therein is present as a discontinuous phase in a continuous oil phase.

In that process it is preferred that the water phase is provided as part of the water-in-oil emulsion so that the monomer may be contacted with the emulsion directly and at the start of the polymerisation process. The magnetic component used in the particles is supplied as a magnetic fluid, most preferably as a ferrofluid, typically having an average particle size of about 8.5nm. The monomer is preferably a silicon oxide or hydroxide.

In accordance with GB0116358.3, a process is provided for the preparation of polymer magnetic particles, which comprises:

(a) providing polymer particles having a porous interior bearing charged groups; and

(b) contacting the polymer particles with a magnetic fluid comprising a homogeneous dispersion of magnetic

particles which bear a charge opposite to that of the charged groups of the porous interior, whereby the magnetic particles are incorporated into the porous interior to produce polymer magnetic particles.

In this process it is preferred that the magnetic fluid is provided as a ferrofluid, most preferably having a size in the range of 1 to less than 10nm to enable the particles to be dispersed in any of the small pores of the polymer particles. Among the methods available for preparing the polymer particles may be mention the use of vinyl monomers, polyvinyl monomers and mixtures thereof.

Depending on the form in which the isolated nucleic acid is required, a further elution step can be provided. In some cases it may be satisfactory for the nucleic acid to remain bound to the magnetic particles. This may be the case if further manipulations of the nucleic acid on a solid phase are required, such as an amplification step. Equally, the nucleic acid may be eluted from the magnetic particles by applying an elution solution, which may simply be water or a buffer.

Detailed Description

The present invention is now described in more detail, by way of example only, with reference to the following Examples.

Example 1

The magnetic particles. Magnetic Silica particles were obtained in accordance with GB0116359.1, filed 4th July 2001 and suspended as 25mg in 100ul isopropanol. To this was added 50ul of 8M LiCl.

The chaotropic lysis and binding solution (L). 130 g Guanidine thiocyanate (Sigma) was added 95 ml 0.1 M TRIS HCl pH 7 (Sigma) + 8 ml 0.5 M EDTA (Invitrogen) and 2.5 g tween-20 (Sigma). The solution was heated on a water bath at 30C for 1 h. To this solution was added 16 ul 5% NH_3 (Merck)/ml chaotropic solution to leave pH at 8.5 as an ammonia or ammonium chaotropic solution.

The chaotropic wash I solution. 120g Guanidine hydrochloride (Sigma) was added water to a total of 160 ml (7.5M). To this solution was added 2.4g Tween-20 (Sigma) and 40 ml 96% EtOH.

The ethanol based wash II solution. To 200µl 2M NaCl (Sigma) was added 700 ul 96% EtOH. To 100µl of this solution was added 200 ul water.

The DNA binding procedure. 350 ul of whole blood (WBC 5.1) were added 720 ul of the chaotropic lysis and binding solution. After 1 min, magnetic silica beads were added (140 ul) and the solution was allowed to incubate for 10 min where after the magnetic were collected on a magnet. The beads were resuspended in washing solution I and again collected on a magnet. This was repeated once. The beads were resuspended and washed in washing solution II and collected on a magnet. Repeated once. The beads were washed

with pure water when still on the magnet. Finally, 200 ul water was added to the beads and they were resuspended at ambient temperature for ca 2 min. The beads were collected on a magnet and the supernatant was transferred to a new tube The yield of isolated DNA was measured on a Spectrophotometer (Perkin Elmer, Lambda EZ 201).

Yield DNA: 6.56 ug (62% of theoretical).

Example 2

The magnetic particles. As example 1.

The chaotropic lysis and binding solution (L). As in example 1.

The chaotropic wash I solution. As example 1

The ethanol based wash II solution. As in example 1.

The DNA binding procedure. 75 ul buffycoat (ca. 10^8 cells/ml) was added as in example 1.

Yield DNA: 11.65 ug.

Example 3

The magnetic particles. The magnetic silica particles of Example 1 were suspended as 23.4 mg in 100 ul 4M LiCl.

The chaotropic lysis and binding solution (L). As in example 1.

The chaotropic wash I solution. 120g Guanidine hydrochloride (Sigma) was added water to a total of 160 ml (7.5M). To solution was added 2.4g Tween-20 (Sigma) and 53 ml isopropanol.

The ethanol based wash II solution. As in example 1.

The DNA binding procedure. As in example 2 except that 100 ul of magnetic silica particles were added.

Yield DNA: 9.69 ug.

Example 4

The magnetic particles. As example 1.

The chaotropic lysis and binding solution (L). 75 ul buffy coat was added as in example 2.

The chaotropic wash I solution. As example 3.

The ethanol based wash II solution. As in example 1.

The DNA binding procedure. 75 ul of buffycoat (ca. 10^8 cells/ml) was added as in example 1.

Yield DNA: 10.94 ug.

Example 5

The magnetic particles. As example 1.

The chaotropic lysis and binding solution (L). As in example 1.

The chaotropic wash I solution. As example 1

The ethanol based wash II solution. As in example 1.

The DNA binding procedure. 50 ul buffy coat (ca. 10^8 cells/ml) was added as in example 2. Instead of using magnetic beads suspended in isopropanol, beads were suspended in 80ul 4M LiCl and 100ul isopropanol was added immediately before mixing with the sample and chaotrope.

Yield DNA: 8 ug.

Example 6

The magnetic particles. As example 1 except isopropanol, n-propanol and ethanol were compared in separate experiments.

The chaotropic lysis and binding solution (L). As in example 1.

The chaotropic wash I solution. As example 1

The ethanol based wash II solution. As in example 1.

The DNA binding procedure. 75 ul buffy coat (ca. 10^8 cells/ml) was added as in example 2.

Yield DNA: isopropanol 8.1 ug; ethanol 8.0 ug; n-propanol 7.6 ug.

Example 7

The magnetic particles. The magnetic silica particles of Example 1 were suspended as 23.4 mg in 100 ul 4M LiCl.

The chaotropic lysis and binding solution (L). As in example 1.

The chaotropic wash I solution. 120g Guanidine hydrochloride (Sigma) was added water to a total of 160 ml (7.5M). To solution was added 2.4g Tween-20 (Sigma) and 53 ml ethanol.

The ethanol based wash II solution. As in example 1.

The DNA binding procedure. As in Example 2 except that 100 ul of magnetic silica particles were added to the chaotropic solution containing the sample (again, 75 ul buffy of ca 1x10(8) white cells/ml). After 2.5 min, ethanol (40, 60, and 100 ul respectively, concentration of ethanol 4, 6 an 10%, respectively) was added and the incubation of sample/beads/chaotropic solution was continued for another 2.5 min where after the nucleic acid/particles ware

collected on a magnet and wash according to example 1. The pellet was nicely dispersed and washed.

Yield DNA: 8.8ug (40 ul ethanol)
8.8ug (60 ul ethanol)
9.4ug (100 ul ethanol)

Example 8 (Comparative)

The magnetic particles. As example 1 except the magnetic silica particles were suspended as 23.4 mg in 100 ul 4M LiCl in the absence of any alcohol.

The chaotropic lysis and binding solution (L). As in example 1.

The chaotropic wash I solution. As example 1

The ethanol based wash II solution. As in example 1.

The DNA binding procedure. 75 ul buffy coat (ca. 10^8 cells/ml) was added as in example 2.

Yield DNA: 6.1 ug.

CLAIMS:

1. A process for isolating nucleic acid from a nucleic acid-containing biological sample, which comprises:

(a) providing magnetic particles capable of binding nucleic acid;

(b) contacting the sample with the magnetic particles so as to bind the nucleic acid thereto;

(c) forming an aggregate of the magnetic particles by the application of a magnetic field, which aggregate comprises magnetic particles with the nucleic acid bound thereto and contaminants from the biological sample;

(d) contacting the aggregate with a washing solution to disperse the aggregate and release the contaminants therefrom; and

(e) separating therefrom the magnetic particles with the nucleic acid bound thereto;

wherein the aggregate is formed in the presence of an amount of a water-miscible lower alcohol sufficient to facilitate dispersion of the aggregate and release of the contaminants therefrom when washed in step (d), with the proviso that, when the magnetic particles are silica-based magnetic glass particles provided with the water-miscible lower alcohol, the water-miscible lower alcohol is part of an aqueous mixture which further comprises a salt.

2. A process for isolating nucleic acid from a nucleic acid-containing biological sample, which comprises:

(a) providing magnetic particles capable of binding nucleic acid;

(b) contacting the sample with the magnetic particles so as to bind the nucleic acid thereto;

(c) forming an aggregate of the magnetic particles by the application of a magnetic field, which aggregate comprises magnetic particles with the nucleic acid bound thereto and contaminants from the biological sample;

(d) contacting the aggregate with a washing solution to disperse the aggregate and release the contaminants therefrom; and

(e) separating therefrom the magnetic particles with the nucleic acid bound thereto;

wherein the aggregate is formed in the presence of an amount of a water-miscible lower alcohol sufficient to facilitate dispersion of the aggregate and release of the contaminants therefrom when washed in step (d), and

wherein substantially no Proteinase K is present to digest protein from the sample, before step (d).

3. A process for isolating nucleic acid from a nucleic acid-containing biological sample, which comprises:

(a) providing magnetic particles capable of binding nucleic acid;

(b) contacting the sample with the magnetic particles so as to bind the nucleic acid thereto;

(c) forming an aggregate of the magnetic particles by the application of a magnetic field, which aggregate comprises magnetic particles with the nucleic acid bound thereto and contaminants from the biological sample;

(d) contacting the aggregate with a washing solution to disperse the aggregate and release the contaminants therefrom; and

(e) separating therefrom the magnetic particles with the nucleic acid bound thereto;

wherein the aggregate is formed in the presence of an amount of a water-miscible lower alcohol sufficient to facilitate dispersion of the aggregate and release of the contaminants therefrom when washed in step (d), and

wherein the water-miscible lower alcohol is provided after or at approximately the same time as step (b).

4. A process according to claim 1 or claim 3, wherein substantially no Proteinase K is present to digest protein.

5. A process according to claim 1, claim 2 or claim 4, wherein at least a part of the water-miscible lower alcohol is provided with the magnetic particles.

6. A process according to claim 5, wherein the magnetic particles are provided as a suspension in an aqueous mixture of the water-miscible lower alcohol.

7. A process according to claim 6, wherein the aqueous mixture further comprises a salt.

8. A process according to claim 7, wherein the salt is at a concentration in the range 0.4M to 8M in the aqueous mixture.

9. A process according to claim 7 or claim 8, wherein the salt comprises lithium chloride.

10. A process according to claim 1, claim 2 or claim 4, wherein at least a part of the water-miscible lower alcohol is provided in a lysis step which comprises subjecting the biological sample to conditions to lyse the sample.

11. A process according to claim 10, wherein the water-miscible lower alcohol is present in the lysis step in an amount in the range from 3 to 40% v/v.

12. A process according to any one of claims 1 to 4, wherein at least a part of the water-miscible lower alcohol is provided when the sample is contacted with the magnetic particles.

13. A process according to claim 12, wherein the water-miscible lower alcohol is present in an amount in the range from 10 to 90% v/v.

14. A process according to any preceding claim, wherein further water-miscible lower alcohol is provided with the washing solution.

15. A process according to claim 14, wherein the further water-miscible lower alcohol is present in an amount in the range 5 to 70% v/v with respect to the washing solution.

16. A process according to any preceding claim, wherein the water-miscible lower alcohol comprises ethanol, isopropanol or n-propanol.

17. A process according to any preceding claim, wherein the washing solution comprises a chaotrope to facilitate washing.

18. A process according to claim 17, which further comprises a further washing step to remove chaotrope.

19. A process according to any preceding claim, wherein the magnetic particles comprise polymer magnetic particles in which the magnetic component thereof is substantially uniformly distributed.

20. A process according to any preceding claim, wherein the nucleic acid comprises DNA.

21. A process according to any one of claims 1 to 19, wherein the nucleic acid comprises RNA.

22. A process according to any preceding claim, wherein the sample is contacted with the magnetic particles in the presence of a chaotrope to facilitate binding of the nucleic acid to the magnetic particles.

23. A process according to any preceding claim, which further comprises a step of eluting the nucleic acid from the magnetic particles.

24. A kit for isolating nucleic acid from a nucleic acid-containing biological sample, which kit comprises:

(a) magnetic particles capable of binding nucleic acid;

(b) a source of a water-miscible lower alcohol; and

(c) a washing solution for use in the presence of the water-miscible lower alcohol for dispersing an aggregate comprising the magnetic particles with nucleic acid bound thereto and contaminants from the biological sample;

wherein the magnetic particles are not the source of the water-miscible lower alcohol when the magnetic particles are silica-based magnetic glass particles unless (i) no Proteinase K is present in the kit, or (ii) the water-miscible lower alcohol is part of an aqueous mixture which further comprises a salt.

25. A kit according to claim 24, wherein no Proteinase K is present.

26. A kit according to claim 24 or claim 25, wherein at least a part of the water-miscible lower alcohol is provided with the magnetic particles.

27. A kit according to claim 26, wherein the magnetic particles are provided as a suspension in an aqueous mixture of the water-miscible lower alcohol.

28. A kit according to claim 27, wherein the aqueous mixture further comprises a salt.

29. A kit according to claim 28, wherein the salt is at a concentration in the range 0.4M to 8M in the aqueous mixture.

30. A kit according to claim 28 or claim 29, wherein the salt comprises lithium chloride.

31. A kit according to any one of claims 24 to 30, which further comprises a lysis solution for lysing the biological sample.

32. A kit according to claim 31, wherein at least a part of the water-miscible lower alcohol is provided in the lysis solution.

33. A kit according to claim 32, wherein the water-miscible lower alcohol is present in an amount in the range 3 to 40% v/v with respect to the lysis solution.

34. A kit according to any one of claims 24 to 33, which further comprises a nucleic acid binding solution.

35. A kit according to claim 34, wherein the nucleic acid binding solution comprises a chaotrope for facilitating binding of the nucleic acid to the magnetic particles.

36. A kit according to claim 34 or claim 35, wherein at least a part of the water-miscible lower alcohol is provided in the binding solution.

37. A kit according to claim 36, wherein the water-miscible lower alcohol is present in an amount in the range from 10 to 90% v/v with respect to the binding solution.

38. A kit according to any one of claims 24 to 30, wherein further water-miscible lower alcohol is provided with the washing solution.

39. A kit according to claim 38, wherein the water-miscible lower alcohol is present in an amount in the range 5 to 70% v/v with respect to the washing solution.

40. A kit according to any one of claims 24 to 39, wherein the water-miscible lower alcohol comprises ethanol, isopropanol or n-propanol.

41. A kit according to any one of claims 24 to 40, wherein the washing solution comprises a chaotrope to facilitate washing.

42. A kit according to claim 41, which further comprises a further washing solution for removing the chaotrope.

43. A kit according to any one of claims 24 to 42, wherein the magnetic particles comprise polymer magnetic particles in which the magnetic component thereof is substantially uniformly distributed.

44. A kit according to any one of claims 24 to 43, which further comprises a solution for eluting the nucleic acid from the magnetic particles.

45. Use of a water-miscible lower alcohol in a process for isolating nucleic acid from a nucleic acid-containing biological sample, which process comprises:

(a) providing magnetic particles capable of binding nucleic acid;

(b) contacting the sample with the magnetic particles so as to bind the nucleic acid thereto;

(c) forming an aggregate of the magnetic particles by the application of a magnetic field, which aggregate comprises magnetic particles with the nucleic acid bound thereto and contaminants from the biological sample;

(d) contacting the aggregate with a washing solution; and

(e) separating therefrom the magnetic particles with the nucleic acid bound thereto;

wherein a sufficient amount of the water-miscible lower alcohol is present in step (c) for the purpose of dispersing the aggregate and releasing the contaminants therefrom when washed in step (d).

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 03/01553

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68 C12N15/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6 270 970 B1 (SMITH CRAIG E ET AL) 7 August 2001 (2001-08-07) examples 14,16-18	1-8, 10-29, 31-45
Y		1-45

Y	US 5 681 946 A (REEVE MICHAEL ALAN) 28 October 1997 (1997-10-28) column 1, line 27 - line 49 column 4 -column 8; example 1	1-45

Y	WO 99 58664 A (WHITEHEAD BIOMEDICAL INST) 18 November 1999 (1999-11-18) page 11 -page 12; claims; example 5	1-45

	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 September 2003	24 09. 2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	C-O GUSTAFSSON /ELY

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 03/01553

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JAMES I. TAYLOR ET AL: "Application of magnetite and silica-magnetite composites to the isolation of genomic DNA" JOURNAL OF CHROMATOGRAPHY A, vol. 890, 2000, pages 159-166, XP002253946 page 161 -page 162; table 2 -----	1-45

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/IB 03/01553

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
US 6270970	B1	07-08-2001	AU	4841500 A	05-12-2000
			CA	2372521 A1	23-11-2000
			EP	1179056 A1	13-02-2002
			JP	2002543980 T	24-12-2002
			WO	0070041 A1	23-11-2000
			US	2002001812 A1	03-01-2002
US 5681946	A	28-10-1997	US	5523231 A	04-06-1996
			AT	121748 T	15-05-1995
			CA	2071846 A1	14-08-1991
			DE	69109275 D1	01-06-1995
			DE	69109275 T2	24-08-1995
			DK	515484 T3	04-09-1995
			EP	0515484 A1	02-12-1992
			WO	9112079 A1	22-08-1991
			JP	2703114 B2	26-01-1998
			JP	5504095 T	01-07-1993
WO 9958664	A	18-11-1999	WO	9958664 A1	18-11-1999
			US	6534262 B1	18-03-2003